

SMOKE INHALATION CARCINOGENESIS STUDIES IN MICE

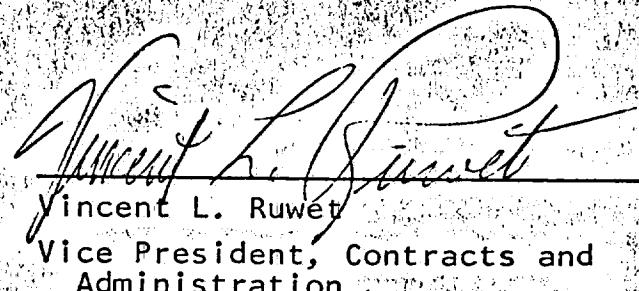
CTR Contract #22
MA Contract 2224

CONTRACT RENEWAL PROPOSAL
(SECOND YEAR)
FOR THE PERIOD

July 1, 1975 - June 30, 1976

1003535837

February 12, 1975


Vincent L. Ruwe
Vice President, Contracts and
Administration

TO: The Council for Tobacco Research U.S.A.
110 East 59th Street
New York, N.Y. 10022

FROM: Microbiological Associates
A Division of Dynasciences Corporation
4733 Bethesda Avenue
Bethesda, Maryland 20014

Prepared by:

Carrie E. Whitmire, Ph.D.
Project Director

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I Progress Report

Since this contract was initiated July 1, 1974 an architectural plan has been drawn, the building contract let and rearrangement of the facilities are well underway. We anticipate the building to be ready for occupancy by June 1, 1975. This will allow a month to prepare the facility for work by July 1, 1975 in the new contract year.

The equipment has been ordered and much of it has begun to arrive. The only item which has given us considerable problems has been the X-ray machine. We have not been able to achieve the desired resolution to provide for sensitive lung cancer diagnosis to date. There are several more approaches to be taken which may allow us to obtain this equipment in the new contract year.

The various studies to define the lung carcinogenesis bioassay system with BaP have been quite encouraging and have been reported on in the progress report for CTR-14.

The dosimetry studies with Oak Ridge National Laboratories has been initiated. A group of studies have been designed and are included in this proposal.

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II Proposal for New Contract Year

A. Smoke Studies

These were outlined in last year's proposal and will be more closely defined before initiated on occupation of the new facilities. At this time various experiments are still in progress which could influence the final experimental design.

B. Dosimetry Studies

Studies have been designed in collaboration with Dr. James Stockely at ORNL and are outlined on the following pages. These studies will be carried out on the Walton Smoking Machine. These data will be used to design dosimetry studies on the new smoking machine to be used in the inhalation studies.

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PROTOCOL FOR MOUSE DOSIMETRY EXPERIMENTS
ON WALTON HORIZONTAL SMOKING MACHINE

Prepared by

C.E. Whitmire
Microbiological Associates

and

J.R. Stokely
Oak Ridge National Laboratory

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These protocols outline proposed collaborative studies by Microbiological Associates and Oak Ridge National Laboratory to investigate mouse dosimetry on the Walton Horizontal Smoking Machine. The objectives of these studies are:

1. To define the dose of tobacco smoke particulates received by mice under selected exposure conditions (exposure time and smoke concentration),
2. To ascertain possible effects of sex and strain on dosimetry so that a rational selection can be made for future studies of biological impact,
3. To determine retention and clearance rate of smoke particulates after exposure, and
4. To determine cumulative dose and long-term retention of smoke particulates under typical exposure regimes.

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Responsibilities of Oak Ridge National Laboratory are the following:

1. Weight and RTD selection of cigarettes.
2. Labeling of cigarettes with ^{14}C tracers.
3. Quality control of cigarettes (determination of nicotine, TPM, tar and ^{14}C delivery of selected radiolabeled cigarettes).
4. Shipping of radiolabeled cigarettes to Microbiological Associates.
5. Construction and testing of apparatus for sampling exposure chamber.
6. Instruction and assistance on operation of smoking machines, sampling apparatus, and cigarette conditioning and handling.
7. Analysis of animal tissues for ^{14}C tracers.
8. Analysis of input Cambridge filter pads for nicotine and ^{14}C tracer and chamber samples for ^{14}C tracer.
9. Calculations to obtain the following results:
 - a. absolute activity (dpm) of tracer in each tissue specimen.
 - b. absolute tar (mg) deposited in each tissue specimen based on tracer deposition.
 - c. percent distribution of activity among various tissue specimens for each animal.
 - d. dose expressed as percentage of smoke input to exposure chamber.

Responsibilities of Microbiological Associates are the following:

1. Calibration of smoking machines (puff volume, puff time, exposure time, purge time).
2. Conditioning and weighing of labeled cigarettes after receipt from Oak Ridge National Laboratory.
3. Collection of samples of smoke input to smoking machines and chamber samples obtained during animal exposures.
4. Animal conditioning.
5. Animal exposure.
6. Animal sacrifice and dissection.
7. Shipping of tissue specimens, input filter pads and chamber samples to Oak Ridge National Laboratory.

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Experiment 1. Effect of Exposure Time

1. One mouse strain: C3H/Anf Cum
2. One sex: female
3. Four exposure time intervals: 10,20,30,40 seconds
4. One exposure concentration: 10%
5. Four tissue samples: skinned head, upper trachea and larynx, lungs and lower half of trachea, stomach and esophagus
6. Number of mice per exposure: 10 (plus 10 scrubs)
7. Number of cigarettes per exposure: 3 (2 for chamber input, 1 for exposure)
8. Chamber samples per exposure: 1
9. Cigarette type: Kentucky Reference 1A1 loaded with 5×10^6 dpm ^{14}C -dotriaccontane--weight and RTD tested (± 20 mg, ± 5 mm H_2O)
10. Number of repetitive exposures: 4 (40 Mice)
11. Instant sacrifice
12. Tissue specimens: 640
13. Total cigarettes: 60 (assume 25% loss).

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Experiment II. Effect of Smoke Concentration

1. One exposure time interval: based on experiment I
2. Five exposure concentrations: 20% (2 cigarettes-384 ml chamber),
30% (3 cigarettes-384 ml chamber),
5% (1 cigarette-768 ml chamber),
10% (2 cigarettes-768 ml chamber),
15% (3 cigarettes-768 ml chamber)
3. Number of cigarettes per exposure: 6(20%), 9(30%), 3(5%),
6(10%), 9(15%)
4. One mouse strain: C3H/Anf Cum
5. One sex: female
6. Four tissue samples: skinned head, upper trachea and larynx, lungs and lower half of trachea, stomach and esophagus
7. Number of mice per exposure: 10 (plus 10 scrubs)
8. Chamber samples per exposure: 1
9. Cigarette type: Kentucky Reference 1A1 loaded with 5×10^6 dpm ^{14}C -dotriacontane--weight and RTD tested (± 20 mg, ± 5 mm H_2O)
10. Number of repetitive exposures: 4 (40 mice)
11. Instant sacrifice
12. Total tissue specimens: 800
13. Total cigarettes: 165 (assume 25% loss)

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Experiment III. Effect of Sex

1. Two sex: male and female
2. One exposure time interval: based on experiment I
3. One exposure concentration: based on experiment II
4. Number of mice per exposure: 20 (10 male, 10 female)
5. Number of repetitive exposures: 4 (40 mice)
6. Four tissue samples: skinned head, upper trachea and larynx, lungs and lower half of trachea, stomach and esophagus
7. Chamber samples per exposure: 1
8. Cigarette type: Kentucky Reference 1A1 loaded with 5×10^6 dpm ^{14}C -dotriacontane--weight and RTD tested (± 20 mg, ± 5 mm H_2O)
9. Instant sacrifice
10. Tissue specimens: 300
11. Total cigarettes: 15 (assume 25% loss).

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Experiment IV: Strain Differences

1. Four mouse strains: C3H/f, C57BL/6, DBA/2, B6C3F1
2. Sex: based on experiment III (male, female, both)
3. One exposure time: based on experiment I
4. One exposure concentration: based on experiment II
5. Four tissue samples: skinned head, upper trachea and larynx, lungs and lower half of trachea, stomach and esophagus
6. Number of mice per exposure: 20 (10 each of 2 strains or sexes)
7. Number of cigarettes per exposure: 3 (2 for chamber input, 1 for exposure)
8. Chamber samples per exposure: 1
9. Cigarette type: Kentucky Reference 1A1 loaded with 5×10^6 dpm ^{14}C -dotriacontane--weight and RTD tested (± 20 mg, ± 5 mm H_2O)
10. Instant sacrifice
11. Tissue specimens: 640 (1 sex)
1280 (2 sexes)
12. Number of cigarettes: 30 (1 sex)
60 (2 sexes) (assume 25% loss)

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Experiment V: Retention Period of ^{14}C -Dotriacontane

1. One mouse strain: C3H/f
2. One sex: based on experiments III and IV
3. One exposure time: based on experiment I
4. One exposure concentration: based on experiment II
5. Five tissue samples per animal: same as experiment I plus composite sample of all other animal tissues--animal skinned--skin not included in composite.
6. Number of mice per exposure: 20
7. Five sacrifice times after smoking: 0.25, 1, 4, 16, 24 hours. Four animals sacrificed at each time.
8. Number of cigarettes per exposure: 3
9. Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C -ditriacontane.
10. Number of repetitive exposures: 4
11. Tissue specimens: 400
12. Number of cigarettes: 15 (assume 25% loss)

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Experiment VI: Retention Period of ^{14}C -Benz(a)pyrene

1. Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C -benz(a)pyrene
2. One mouse strain: C3H/f or B6C3F1
3. One sex: based on experiments III and IV
4. One exposure time: based on experiment I
5. One exposure concentration: based on experiment II
6. Five tissue samples per animal: same as experiment I plus composite sample of all other animal tissues--animal skinned--skin not included in composite.
7. Number of mice per exposure: 20
8. Five sacrifice times after smoking: 0.25, 1, 4, 16, 24 hours. Four animals sacrificed at each time.
9. Number of cigarettes per exposure: 3
10. Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C -ditriacontane.
11. Number of repetitive exposures: 4
12. Tissue specimens: 400
13. Number of cigarettes: 15 (assume 25% loss)

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Experiment VII: Retention Period of ^{14}C -Nicotine

1. Five sacrifice times after smoking: immediately, 0.25, 0.5, 1, 4 hours after smoking. Four animals sacrificed at each time.
2. Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C -nicotine.
3. One mouse strain: C3H/f
4. One sex: based on experiments III and IV
5. One exposure time: based on experiment I
6. One exposure concentration: based on experiment II
7. Five tissue samples per animal: same as experiment I plus composite sample of all other animal tissues-- animal skinned--skin not included in composite.
8. Number of mice per exposure: 20
9. Five sacrifice times after smoking: 0.25, 1, 4, 16, 24 hours. Four animals sacrificed at each time.
10. Number of cigarettes per exposure: 3
11. Cigarette type: 1A1 loaded with maximum amount (up to 1×10^8 dpm) ^{14}C -dotriacontane.
12. Number of repetitive exposures: 4
13. Number of cigarettes: 15 (assume 25% loss).

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Experiment VIII: Comparative Retention Periods for Two
Mouse Strains

1. Two strains: based on experiment IV
2. One sex: based on experiments III and IV
3. One exposure time: based on experiment I
4. One exposure concentration: based on experiment II
5. Two retention periods: based on experiments V, VI, and VII
6. Five tissue specimens per animal: experiment V
7. Twenty mice per exposure: ten mice sacrificed at each retention period
8. Number of repetitive exposures: 2
9. Three type cigarettes: Kentucky Reference 1A1 loaded with ^{14}C -nicotine, ^{14}C -benz(a)pyrene, or ^{14}C -dotriacontane (maximum load)
10. Tissue specimens: 1200
11. Number of cigarettes: 15 (assume 25% loss).

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Experiment IX: Cumulative Dose and Long Term Retention

1. Number of radiolabeled cigarettes given per 8 hour day: 2, 5, 10, 20
2. One strain - based on experiment VIII
3. Four animals sacrificed at each of the following times after exposure: immediately, 4, 24, 48, 120 hours
4. Three type cigarettes: Kentucky Reference 1A1 loaded with ^{14}C -nicotine, ^{14}C -benz(a)pyrene, or ^{14}C -dotriacontane (maximum load) or all three at one time.
5. Number of repetitive tests: 1
6. Other conditions: same as experiment VIII
7. Tissue specimens: 1200
8. Number of cigarettes: 169 (assume 25% loss).

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Summary of Tissue Specimens and Number of Cigarettes

<u>Experiment</u>	<u>Tissue Specimens¹</u>	<u>Number of Cigarettes²</u>
I	640	60
II	800	165
III	320	15
IV	1280	60
V	400	15
VI	400	15
VII	400	15
VIII	1200	15
IX	1200	169
	6640	529

1 Maximum

2 Assume 25% loss

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III Budget

The budget has been divided into the various support and scientific sections required to carry out the proposed studies. Several items of equipment have been included in this year's budget which will be needed to carry out the program.

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BUDGET CTR-22 (July 1, 1975 - June 30, 1976)

	<u>Section</u>					
	<u>A*</u>	<u>B</u>	<u>C</u>	<u>D</u>	<u>E</u>	<u>Totals</u>
A. Direct Labor (Schedule A)	\$ 23,240	\$ 20,234	\$ 29,573	\$ 54,509	\$110,645	\$238,201
B. Overhead (115% of A)	26,726	23,269	34,009	62,685	127,242	273,931
C. Other Direct Costs	--	7,000	35,000	10,000	45,000	97,000
D. Travel	<u>2,000</u>	<u>--</u>	<u>--</u>	<u>1,500</u>	<u>--</u>	<u>3,500</u>
E. Total (A-D)	51,966	50,503	98,582	128,694	282,887	612,632
F. G & A (16% of E)	<u>8,315</u>	<u>8,080</u>	<u>15,773</u>	<u>20,591</u>	<u>45,262</u>	<u>98,021</u>
G. Total	60,281	58,583	114,355	149,285	328,149	710,653
H. Fixed Fee	<u>6,698</u>	<u>6,509</u>	<u>12,705</u>	<u>16,586</u>	<u>36,457</u>	<u>78,955</u>
I. Total Before Equipment and Overtime Premium	66,979	65,092	127,060	165,871	364,606	789,608
J. Equipment	1,500	300	8,000	5,500	10,000	25,300
K. Overtime Premium	--	--	--	1,500	--	1,500
L. Total	<u>\$ 68,479</u>	<u>\$ 65,392</u>	<u>\$135,060</u>	<u>\$172,871</u>	<u>\$374,606</u>	<u>\$816,408</u>
M. Contingency						<u>83,592</u>
N. Total Cost						<u>\$900,000</u>

*Sections: A. Administration
B. Data Processing
C. Dosimetry with ORNL

D. Histopathology
E. Inhalation Studies

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SCHEDULE A - Direct Labor For CTR Contract 22
 (July 1, 1975 - June 30, 1976)

<u>Section/Personnel</u>	<u>\$/Hr.</u>	<u>Time</u>	<u>Hours</u>	<u>\$</u>
A. Administration				
C.E. Whitmire, Ph.D.	14.38	50	963	\$13,848
C.F. Demoise, Ph.D.	8.18	25	482	3,943
Vacancy, Adm. Assist.	4.08	50	963	3,929
			<u>2,408</u>	<u>\$21,720</u>
			7% Merit Raise	1,520
				<u>\$23,240</u>
B. Data Processing				
M. Haven, M.S.	10.58	50	963	\$10,189
P. Gradwell, Data Tech.	3.84	30	578	2,220
Vacancy, Data Tech.	3.50	50	963	3,371
B. Ross, Key Punch Tech.	3.25	50	963	3,130
			<u>3,467</u>	<u>\$18,910</u>
			7% Merit Raise	1,324
				<u>\$20,234</u>
C. Dosimetry Studies with ORNL				
D. Dansie, Tech.	3.80	100	1,926	\$ 7,319
Vacancy, Tech.	4.20	100	1,926	8,089
G. Gomez, Jr. Tech.	3.25	100	1,926	6,259
J. Fernandez, Anim. Care.	3.10	100	1,926	5,971
			<u>7,704</u>	<u>27,638</u>
			7% Merit Raise	1,935
				<u>\$29,573</u>
D. Histopathology				
Vacancy, D.V.M.	17.30	100	1,926	\$33,320
S.A. Gosnell, Super. Tech.	4.30	50	963	4,141
Vacancy, Tech.	3.50	100	1,926	6,741
Vacancy, Tech.	3.50	100	1,926	6,741
			<u>6,741</u>	<u>\$50,943</u>
			7% Merit Raise	3,566
				<u>\$54,509</u>
E. Inhalation Studies				
7 Vacancies, Jr. Tech.	3.50	100	13,482	\$47,187
4 Vacancies, Jr. Tech.	3.50	50	3,852	13,482
A. Saborit, Washroom Aide	3.39	100	1,926	6,529
2 Vacancies, Washroom Aide	3.20	100	3,852	12,326
1 Vacancy, Anim. Care.	3.35	100	1,926	6,452
A. Lopez, Sr. Tech.	4.80	100	1,926	9,245
G. Griffin, Tech.	4.25	100	1,926	8,187
			<u>28,890</u>	<u>\$103,407</u>
			7% Merit Raise	7,238
				<u>\$110,645</u>
Totals		25.5 Man Years	<u>49,210</u>	<u>\$238,201</u>

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Schedule B: CTR-22

(July 1, 1975 - June 30, 1975)

Desks, files	\$ 450
Typewriter	650
2 calculators	400
Computer files	300
Gas chromatographic and other dosimetry monitoring equipment	8,000
Pathologist's microscope tables, etc.	1,500
Microprojector	4,000
X-ray machine and safety shield	<u>10,000</u>
	\$25,300

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